DOCKET NO.: CARP-0123 PATENT

Application No.: 10/579,981

Preliminary Amendment - First Action Not Yet Received

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (currently amended) A transformed host cell containing comprising

- i) a chromosomal gene which inhibits cell growth; and
- ii) a plasmid encoding an antisense sequence,

wherein the antisense sequence encoded by the plasmid inhibits the action of the chromosomal gene, thereby permitting cell growth.

- 2. (original) A transformed host cell according to claim 1 wherein the plasmid comprises a cloning site for insertion of a gene of interest.
- 3. (currently amended) A transformed host cell according to elaim 1 or claim 2, wherein the plasmid further comprises a gene of interest.
- 4. (currently amended) A transformed host cell of any one of claims 1-3 according to claim 1, wherein the antisense sequence encoded by the plasmid inhibits the action of the chromosomal gene by binding to the chromosomal gene.
- 5. (currently amended) A transformed host cell of claim any one of claims 1-3 according to claim 1, wherein the antisense sequence encoded by the plasmid inhibits the action of the chromosomal gene by binding to mRNA transcribed from the chromosomal gene.
- 6. (currently amended) A transformed host cell of any one of claims 1-5 according to claim 1, wherein a regulatory sequence is operably linked to the chromosomal gene.
- 7. (currently amended) A transformed host cell of according to claim 6, wherein the antisense sequence encoded by the plasmid inhibits the action of the chromosomal gene by binding to the regulatory sequence.
- 8. (currently amended) A transformed host cell of according to claim 6, wherein the antisense sequence encoded by the plasmid inhibits the action of the chromosomal gene by binding to mRNA transcribed from the regulatory sequence.

Application No.: 10/579,981

Preliminary Amendment - First Action Not Yet Received

9. (currently amended) A transformed host cell of according to claim 8, wherein the

PATENT

antisense sequence encoded by the plasmid is encoded by the origin of replication of the

plasmid.

10. (original) A transformed host cell according to claim 9, wherein the antisense

sequence encoded by the plasmid is RNAI or a portion thereof and the regulatory sequence

operatively linked to the chromosomal gene encodes RNAII or a portion thereof.

11. (currently amended) A transformed host cell according to claim 9, wherein

the antisense sequence encoded by the plasmid is RNAII or a portion thereof and the

regulatory sequence operatively linked to the chromosomal gene encodes RNAI or a portion

thereof.

12. (original) A host cell comprising a chromosomal gene which inhibits cell growth

operatively linked to a regulatory sequence located upstream of the chromosomal gene,

wherein the regulatory sequence is an RNAI gene or a portion thereof, or an RNAII gene or a

portion thereof.

13. (currently amended) A transformed host cell according to any one of claims 1-11

claim 1 or a host cell according to claim 12, wherein the cell is in culture in vitro.

14. (currently amended) A transformed host cell according to any one of claims 1-11

claim 1 or a host cell according to claim 12 and 13 which is a prokaryotic cell.

15. (currently amended) A transformed host cell or a host cell according to claim 14

which is a bacterial cell.

16. (currently amended) A transformed host cell or a host cell according to claim 15,

wherein the cell is a gram negative bacterial cell.

17. (currently amended) A transformed host cell or a host cell according to claim 16,

wherein the cell is an E. coli cell or a Salmonella cell.

Application No.: 10/579,981

Preliminary Amendment - First Action Not Yet Received

18. (currently amended) A transformed host cell or a host cell according to claim 15, wherein the cell is a gram positive bacterial cell.

PATENT

- 19. (currently amended) A transformed host cell or a host cell according to claim 18, wherein the cell is a *Bacillus* cell.
- 20. (currently amended) A transformed host cell or a host cell according to any one of claims 15-19 claim 15 which is an attenuated cell.
- 21. (currently amended) A transformed host cell or host cell according to any one of claims 1-13 claim 1 wherein the cell is a eukaryotic cell.
- 22. (currently amended) A transformed host cell according to claim 21 wherein the cell is a fungi, such as yeast.
- 23. (original) A transformed host cell according to claim 21, wherein the cell is a plant cell.
- 24. (currently amended) A transformed host cell according to claim 21 wherein the cell is an animal cell such as a mammalian cell or an insect cell.
- 25. (currently amended) A transformed host cell or a host-cell according to any one of claims 1-24 claim 1, wherein the chromosomal gene is a toxin gene.
- 26. (currently amended) A transformed host cell or a host cell according to claim 25, wherein the toxin gene is sacB.
- 27. (currently amended) A transformed host cell or a host cell according to any one of claims 1-24 claim 1, wherein the chromosomal gene encodes a repressor protein that inhibits expression of a second chromosomal gene essential for cell growth.
- 28. (currently amended) A transformed host cell or a host cell according to claim 27, wherein the second chromosomal gene is conditionally essential for cell growth.

Application No.: 10/579,981

Preliminary Amendment - First Action Not Yet Received

29. (currently amended) A transformed host cell or a host cell of according to claim 27 or claim 28 wherein the chromosomal gene encodes the repressor *lacI* and the second

PATENT

chromosomal gene is operatively linked to a lac operator and promoter.

30. (currently amended) A transformed host cell or host cell according to claim 27 or

claim 28 wherein the chromosomal gene is dapD or fabA.

31. (currently amended) A transformed host cell or host cell according to any one of

claims 1-24 claim 1, wherein the chromosomal gene encodes an antisense sequence that

inhibits expression of a second chromosomal gene essential for cell growth.

32. (currently amended) A transformed host cell or host cell according to claim 31,

wherein the antisense sequence encoded by the chromosomal gene inhibits expression of the

second chromosomal gene by binding to the chromosomal gene.

33. (currently amended) A transformed host cell or host cell according to claim 31,

wherein the antisense sequence encoded by the chromosomal gene inhibits expression of the

second chromosomal gene by binding to mRNA transcribed from the second chromosomal

gene.

34. (currently amended) A transformed host cell or host cell according to any one of

claims 31-33 claim 31 wherein the second chromosomal gene is conditionally essential for

cell growth.

35. (currently amended) A transformed host cell or host cell according to any one of

claims claim 31-33 claim 31, wherein the second chromosomal gene is dapD or fabA.

36. (currently amended) A transformed host cell or host cell according to any one of

claims 1-35 claim 1 wherein the chromosomal gene or the regulatory sequence chromosomal

gene fusion is under the control of a constitutive promoter.

37. (currently amended) A transformed host cell or host cell according to any one of

claims 1-35 claim 1 wherein the chromosomal gene, or the regulatory sequence chromosomal

gene fusion is under the control of an inducible promoter.

DOCKET NO.: CARP-0123 PATENT

Application No.: 10/579,981

Preliminary Amendment - First Action Not Yet Received

38. (currently amended) A method of maintaining a plasmid in a host cell in vitro

comprising the step of culturing a transformed host cell of any one of claims 1-11

or 13-37 according to claim 1 under conditions sufficient to permit said cell to grow.

39. (currently amended) A method of producing plasmid DNA comprising culturing a

transformed host cell according to the method of elaim 40 claim 38 and isolating the plasmid

DNA.

40. (currently amended) A method of producing a recombinant protein comprising

culturing a transformed host cell comprising a plasmid encoding a protein of interest

according to the method of claim 40 claim 38 and isolating the protein from the cell.

41. (currently amended) A pharmaceutical composition comprising a transformed host

cell or a host cell according to any one of claims 1 to 37 claim 1 together with a

pharmaceutically acceptable excipient, diluent or buffer.

42. (canceled)

43. (canceled)

44. (currently amended) A method of delivering a gene of interest to a patient comprising

administering to the patient a transformed host cell according to any one of claims 3 to 11 or

13 to 37 claim 3.

45. (currently amended) A method of maintaining a plasmid in a recipient organism

comprising introducing a transformed host cell according to any one of claims 1 to 11 or 13

to 37 claim 1 into said organism, wherein said chromosomal gene is in said transformed host

cell is essential for cell growth in vivo.

46. (new) A host cell according to claim 12, wherein the cell is in culture in vitro.

47. (new) A host cell according to claim 12 which is a prokaryotic cell.

48. (new) A host cell according to claim 47 which is a bacterial cell.

Page 6 of 10

PATENT

DOCKET NO.: CARP-0123 **Application No.:** 10/579,981

Preliminary Amendment - First Action Not Yet Received

- 49. (new) A host cell according to claim 48, wherein the cell is a gram negative bacterial cell.
- 50. (new) A host cell according to claim 49, wherein the cell is an *E. coli* cell or a *Salmonella* cell.
- 51. (new) A host cell according to claim 48, wherein the cell is a gram positive bacterial cell.
- 52. (new) A host cell according to claim 51, wherein the cell is a *Bacillus* cell.
- 53. (new) A host cell according to claim 47 which is an attenuated cell.
- 54. (new) A host cell according to claim 12 wherein the cell is a eukaryotic cell.
- 55. (new) A host cell according to claim 54, wherein the cell is a fungi.
- 56. (new) A host cell according to claim 54, wherein the cell is a plant cell.
- 57. (new) A host cell according to claim 54, wherein the cell is an animal cell.
- 58. (new) A host cell according to claim 12, wherein the chromosomal gene is a toxin gene.
- 59. (new) A host cell according to claim 58, wherein the toxin gene is sacB.
- 60. (new) A host cell according to claim 12, wherein the chromosomal gene encodes a repressor protein that inhibits expression of a second chromosomal gene essential for cell growth.
- 61. (new) A host cell according to claim 60, wherein the second chromosomal gene is conditionally essential for cell growth.
- 62. (new) A host cell of claim 60, wherein the chromosomal gene encodes the repressor *lacI* and the second chromosomal gene is operatively linked to a *lac* operator and promoter.

Application No.: 10/579,981

Preliminary Amendment - First Action Not Yet Received

63. (new) A host cell according to claim 60, wherein the chromosomal gene is dapD or

PATENT

fabA.

64. (new) A host cell according to claim 12, wherein the chromosomal gene encodes an

antisense sequence that inhibits expression of a second chromosomal gene essential for cell

growth.

65. (new) A host cell according to claim 64, wherein the antisense sequence encoded by

the chromosomal gene inhibits expression of the second chromosomal gene by binding to the

chromosomal gene.

66. (new) A host cell according to claim 64, wherein the antisense sequence encoded by

the chromosomal gene inhibits expression of the second chromosomal gene by binding to

mRNA transcribed from the second chromosomal gene.

67. (new) A host cell according to claim 64, wherein the second chromosomal gene is

conditionally essential for cell growth.

68. (new) A host cell according to claim 64, wherein the second chromosomal gene is

dapD or fabA.

69. (new) A host cell according to claim 12, wherein the chromosomal gene or the

regulatory sequence-chromosomal gene fusion is under the control of a constitutive promoter.

70. (new) A host cell according to claim 12, wherein the chromosomal gene, or the

regulatory sequence-chromosomal gene fusion is under the control of an inducible promoter.

71. (new) A pharmaceutical composition comprising a host cell according to claim 12

together with a pharmaceutically acceptable excipient, diluent or buffer.

72. (new) A method of immunizing a patient against a disease caused by a pathogen

comprising administering a transformed host cell according to claim 1 to the patient.

Application No.: 10/579,981
Preliminary Amendment - First Action Not Yet Received

73. (new) A method of immunizing a patient against a disease caused by a pathogen comprising administering a host cell according to claim 12 to the patient.

PATENT